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RAT ISLET ISOGRAFT FUNCTION

EFFECT OF GRAFT VOLUME AND TRANSPLANTATION SITE[1]

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Islet isograft function was analyzed after transplantation of 4 well-defined endocrine volumes (12.5%, 25%, 50%, and 100% of the endocrine volume in the normal adult rat pancreas) to 3 different sites (kidney, liver, and spleen). Graft function was tested in unanesthetized, unstressed rats by the responses to glucose infusion and to a meal. All animals with grafts ≥ 25% of the endocrine volume of the rat pancreas returned to normoglycemia after transplantation. The minimal graft volume for restoring normoglycemia is probably between 12.5% and 25%, since also a small number of grafts of 12.5% were successful. At 1 month, basal glucose and insulin levels were similar to those of controls in rats with grafts to the spleen, but higher in rats with grafts to the kidney or liver. Irrespective of the transplantation site, recipients had higher glucose and lower insulin levels than controls in response to glucose infusion. In response to a meal, however, only the first-phase insulin response was reduced, but the total insulin output during the entire test was similar to that of controls. Graft performance was found to be graft-size dependent. Results of tests performed at 2 months showed a tendency of increasing responsiveness compared with the results of tests at 1 month.

Transplantation of islets of Langerhans has been performed successfully in diabetic rodents (*1, 2*), large laboratory animals (*3–5*), and also in man (*6*). Although normalization of basal glucose levels can be readily achieved by isogenic islet transplantation to various sites in the rodent model, the minimal amount of islet tissue reportedly required to obtain normoglycemia varies considerably (*7, 8*). Furthermore, normalization of glucose tolerance has been observed by some investigators (*9*) but not by others (*10–12*). These inconsistent results can be attributed to differences in the suitability of the transplant sites for islet transplantation (*11*), to the time elapsed after transplantation, and to differences in graft size when the amount of islet tissue is expressed in number of islets instead of endocrine volume. The latter is generally considered to be a more accurate measure of graft size (*13*).

The present study was undertaken to clarify the relation among isograft transplantation site, graft volume, and function in rats. This was performed by transplanting grafts of 4 different defined endocrine volumes to the kidney capsule, the liver, and the spleen. The graft function was tested by measuring the responses to an intravenous glucose tolerance test (IVGTT)* and to a meal in unstressed, freely moving rats (*12, 14–16*). To investigate the eventual changes in time of the graft function, both tests were performed twice, i.e., 1 and 2 months after transplantation.

MATERIALS AND METHODS

Experimental design. We used isogenic transplants to exclude the effect of graft rejection on transplant function. Grafts consisted of clean islets, i.e., without obvious contamination of exocrine tissue, lymph nodes, vessels and ducts. Graft endocrine volumes studied were 1.25, 2.5, 5, and 10 µl, which corresponds to 12.5, 25, 50, and 100%, respectively, of the endocrine volume in the normal rat pancreas, as determined by point count morphometry (*17, 18*). The similarity of 10-µl islet tissue to the endocrine volume of the normal rat pancreas has been confirmed by insulin content determination (*12*).

Islets were transplanted to the liver, spleen, or kidney in streptozotocin-induced diabetic rats. Transplantation was considered successful when nonfasting blood glucose levels below 8.4 mmol/L were reached within 3 weeks after transplantation. Posttransplant normoglycemia was verified to be graft dependent by nephrectomy or splenectomy after islet transplantation to the kidney capsule or the spleen, respectively. For obvious reasons, this could not be verified by removal of the graft after transplantation to the liver. In addition, when the animals with grafts to the liver or spleen were killed, a biopsy was taken of the native pancreas and the liver was removed for histology. Graft function was tested by an IVGTT and by a meal test in unanesthetized and freely moving rats at 2 posttransplant intervals. The results were compared with the results of similar tests performed in normal control rats.

Animals and induction of diabetes. Male inbred Albino Oxford (AO/G) rats were obtained from the Central Animal Laboratory of our medical faculty. Body weights of graft recipients, before the induction of diabetes, ranged from 290 to 310 g. The body weight of islet donors was approximately 350 g. Diabetes was induced by an intravenous injection of streptozotocin (70 mg/kg body weight, Zanosar, a gift from Upjohn Co., Kalamazoo, MI). Rats were considered diabetic when 3 or more consecutive nonfasting blood glucose levels were found exceeding 20 mmol/L over a period of at least 2 weeks before transplantation. During the transplantation procedure, a biopsy of the native pancreas was taken for the histological confirmation of the absence of B cells, defined as < 5% of normal animals. After transplantation, the rats were housed separately for the duration of the experiments (light schedule: lights on from 6:00 a.m. to 6:00 p.m.). Animals had free access to water and standard rat chow (containing 53% carbohydrates, 20% protein, 5% fat, and 22% other constituents [minerals, cellulose, water]), except for the last 2 hr before glucose tolerance testing.

* Abbreviations: AUC-ins (0–20), area under the curve for the first 20 min of testing; IVGTT, intravenous glucose tolerance test.

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Islet isolation. The rat islet isolation method as currently applied in our laboratory has been described previously (*19*). Briefly, the pancreas was distended by intraductal injection of 10 ml of Krebs-Ringer solution containing 25 mM HEPES and 10% BSA (*20*). The pancreas was then excised and cut into small pieces with a pair of scissors. A 2-stage collagenase (Sigma type XI, 2200 U/mg; Sigma Chemical Co., St. Louis, MO) digestion was performed at 37°C (collagenase concentration 1.2 mg/ml and 0.7 mg/ml, respectively).

Islets were separated from the exocrine tissue using a discontinuous dextran gradient (industrial grade, m.w. 70,000; Sigma). Further purification of the islets was obtained by handpicking pure islets, i.e., islets without obvious adherent nonendocrine tissue (< 5% verified by histology), to eliminate nonseparated lymph nodes and vascular and ductal tissue from the islet grafts (*19*).

Islets were identified with the aid of a dissection microscope and a fluorescent lamp (31–28–06 and 31–33–66; Bausch and Lomb, New York, NY). With this illumination, rat islets appear as distinct ochreous bodies, whereas lymph nodes and exocrine tissue are grey. The reliability of this method has been confirmed by histology and dithizone staining.

Preparation of the islet grafts. The total islet volume obtained by the isolation procedure was determined by measuring the islet diameters (*13, 21*), expressed as the mean of 2 axes, of islets in a 5% aliquot of the islet suspension. Subsequently, the total islet volume was calculated, assuming the islets to be perfect spheres. Grafts were prepared by taking an appropriate portion of the islet suspension such as to contain 1.25, 2.5, 5, or 10 µl of endocrine tissue. Transplantations were performed in a random fashion, both with respect to the graft sizes as well as to the graft sites studied, in order to exclude the influence of possible differences in quality of the isolation procedures.

Islet transplantation. Transplantation was performed immediately after the islet isolation. Transplantations into the liver or spleen were performed by direct puncture with a 23G butterfly needle in the portal vein or splenic parenchyma, respectively. During infusion into the spleen, the splenic pedicle was manually occluded to reduce possible islet loss to the liver (*22*). Transplantation under the kidney capsule was performed at the upper pole by carefully expelling the islets from a polyethylene tube introduced at the lower pole of the kidney. After the transplantation was completed, the syringes, butterfly needles, and polyethylene tubes were examined to confirm that all islets had been transplanted.

The number of transplantations performed to the kidney, liver, and spleen were, respectively, 6, 6, and 6 for 10-µl grafts, 6, 10, and 8 for 5-µl grafts, 6, 10, and 6 for 2.5-µl grafts, and 5, 6, and 6 for 1.25-µl grafts.

Transplantation was considered successful when nonfasting blood glucose levels below 8.4 mmol/L (mean + 2×SD of normal controls) were achieved within 3 weeks after transplantation. Glucose concentrations were determined with glucose test strips (Reflotest; Boehringer Mannheim, Germany) 3 times weekly in blood samples taken from the tail vein under light ether anesthesia. The duration of posttransplant hyperglycemia was calculated assuming a linear decrease in time of the blood glucose level between the last determination before and the first determination of a glucose level below 8.4 mmol/L.

Glucose tolerance tests. Glucose tolerance was tested by an IVGTT and by the response to a meal. Both tests were performed at 1 month (4–5 weeks) and at 2 months (8–10 weeks) after transplantation. Experimental and control rats were provided with a cardiac catheter implanted via the right jugular vein for blood sampling. A second catheter was implanted into the left jugular vein for infusion of glucose. This technique allows frequent blood sampling and continuous infusion of fluids in unanesthetized and freely moving rats (*14–16*). Blood lost due to the sampling procedure was replaced by transfusing blood from a normal donor rat at regular intervals. Cannulations were performed 1 week before the first test so that the animals were able to recover from surgery.

IVGTTs were performed by intracardiac infusion of 200 mg of glucose at a rate of 10 mg/min. With this technique, the unphysiological high peak glucose levels, as seen after a bolus injection, are avoided and glucose profiles follow a more physiological pattern, which is more similar to those observed after a test meal (*15*). Blood samples were taken at 10 min and immediately before glucose administration to determine basal values. Subsequent samples were taken at 1, 2, 3, 5, 10, 15, 20, 25, and 30 min after the beginning of the glucose infusion.

For meal tests, basal values were also determined in 2 blood samples. Two grams of rat chow, mixed with 2 ml of water, were then put into the cage. The animals had previously been habituated to ingesting the presented food within 5 min. Blood samples were taken 1, 2, 3, 5, 10, 15, 20, 25, 30, 40, and 50 minutes after the animals started to eat.

Glucose tolerance tests could not be performed in all recipient animals either for technical reasons, such as death caused by the cannulation surgery, or nonpatency of the catheters, or due to the refusal to eat more than 75% (as determined by weight) of the offered test meal within 5 min. The number of animals that were included in the analyses is given in the Tables.

Glucose concentrations were determined in whole blood by a ferricyanide method with a Technicon autoanalyzer. Plasma insulin was measured by RIA, using rat insulin as a standard.

Basal glucose levels are the mean glucose level of the first 2 samples of each animal during glucose tolerance testing, i.e., immediately before glucose infusion or food ingestion. The glucose response to an IVGTT or to a meal test is presented as the absolute glucose values at 20 min and as the incremental glucose level. The total amount of insulin secreted during both tests was quantified by calculating the area under the curve and above baseline for the first 20 min of testing (AUC-ins (0–20)).

Statistical analyses. Results are expressed as the mean ± SEM. Comparison of the transplant success rate was performed by applying Fishers' exact probability test. Correlation coefficients were determined for the relationship between the graft volume and the total amount of insulin secreted. Statistical analysis of all other results was performed using the Mann-Whitney U test. A P-value < 0.05 was considered statistically significant.

RESULTS

Transplant success. In all animals with 10-µl islet grafts and in almost all animals with 5- and 2.5-µl islet grafts, nonfasting normoglycemia was restored within 3 weeks after transplantation (Table 1). The rate of success with these graft volumes was similar, irrespective of the transplantation site (Fig. 1). For all transplant sites, the success rate of the 1.25-µl islet grafts was significantly lower than for grafts comprising a larger endocrine volume. Only 20%, 33%, and 0% of the animals with grafts to the kidney, liver, and spleen, respectively, returned to normoglycemia within 3 weeks after transplantation. In view of the small number of animals involved, we have not analyzed the graft function in response to glucose tolerance testing of animals with 1.25-µl functioning grafts.

TABLE 1. Time (days) after transplantation till normoglycemia was achieved

Transplant site	Graft size[a]			
	1.25 µl	2.5 µl	5 µl	10 µl
Kidney	10	7.5±1.1	4.6±0.7	4.5±0.6
Liver	9, 14	5.6±0.8	3.5±0.4	4.0±0.5
Spleen	—	7.5±1.3[b,c]	4.3±0.8[b]	3.0±0.3[c]

[a] Values are mean ± SEM. Significantly larger for 2.5-µl grafts versus [b]5-µl and [c]10-µl grafts to the spleen (P<0.05).

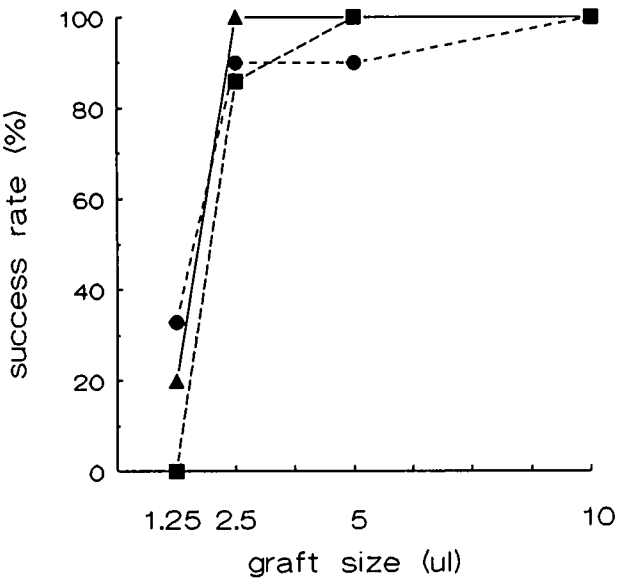

FIGURE 1. Transplant success rate, expressed as the percentage of successful grafts of all transplanted animals. Significantly lower success rates of 1.25-µl graft volume versus 2.5-µl, 5-µl, and 10-µl graft volume after transplantation to kidney (▲), liver (●), and spleen (■), respectively.

The restoration of normoglycemia after transplantation tended to take less time with increasing graft size (Table 1). In rats with islet grafts to the spleen, normoglycemia was achieved significantly earlier by 10-µl grafts than by 2.5-µl grafts. In rats with grafts to the kidney and liver, this period did not differ significantly for animals with either 10-, 5-, or 2.5-µl grafts. In general, normoglycemia was restored earlier in animals with grafts to the liver than in animals with similar graft sizes to the kidney and spleen, but the differences in time between the sites were not statistically significant for each graft size.

Normoglycemia was confirmed to be graft dependent by the return of hyperglycemia after nephrectomy in all animals with grafts to the kidney. Of all 16 animals with grafts to the spleen, 9 became hyperglycemic after splenectomy, but persistent normoglycemia was observed in the other 7. This could not be readily explained by regenerating B cells in the ^{14}native pancreas, since the numbers of granulated B cells in the native pancreas were similar to those in animals that had become hyperglycemic after splenectomy or nephrectomy, and similar to those in animals with grafts to the liver. In 4 of the 7 animals with persistent normoglycemia after splenectomy, a few viable islets were found in the histological sections of the liver, which must have escaped from the splenic site during implantation (*22*). However, the following considerations indicate that these islets have only marginally contributed to the measured splenic islet graft function. First, their number was very low, since their demonstration required extensive sectioning of the liver, whereas the islets of the grafts into the liver were always easily found. Second, a few viable islets were also present in sections of the livers of 2 animals with grafts to the spleen that had become hyperglycemic after splenectomy. Finally, we have found significant differences in the functional parameters between animals with grafts to the spleen and to the liver, but not between animals with grafts to the spleen that showed either hyperglycemia or persistent normoglycemia after splenectomy.

TABLE 2. Basal glucose levels and glucose levels at 20 min during IVGTT (mmol/L) at 1 month after transplantation[a]

Transplant site	Graft size		
	2.5 µl	5 µl	10 µl
Kidney			
Basal	5.6±0.2[b]	5.7±0.2[b]	6.1±0.2[b,f]
20 min	12.0±0.6[b]	10.5±0.7[b]	11.3±0.4[b,h]
n	5	6	4
Liver			
Basal	6.0±0.3[b]	5.6±0.2[b]	5.8±0.2[b,g]
20 min	11.4±0.9[b]	10.4±0.5[b]	11.0±0.8[b]
n	8	8	5
Spleen			
Basal	6.0±0.3[b]	5.8±0.2[b,c]	5.0±0.1[c,f,g]
20 min	12.9±0.6[b,d,e]	10.7±0.4[b,c]	9.7±0.4[b,e,h]
n	5	5	5

[a] In controls, basal glucose levels and glucose levels at 20 min are 5.0±0.1 and 8.3±0.2 mmol/L, respectively (n=11). Values are mean ± SEM.
[b] Significantly ($P<0.05$) higher than controls.
[c–h] Significantly different ($P<0.05$) among the indicated groups.

TABLE 3. Basal glucose levels and glucose levels at 20 min during the meal test (mmol/L) at 1 month after transplantation[a]

Transplant site	Graft size		
	2.5 µl	5 µl	10 µl
Kidney			
Basal	5.6±0.2[b]	5.7±0.2[b]	5.7±0.1[b]
20 min	7.3±0.3[b]	7.1±0.3[b]	7.4±0.1[b,d]
n	6	6	4
Liver			
Basal	5.6±0.3[b]	5.5±0.3[b]	5.4±0.2[b]
20 min	7.2±0.2[b]	6.5±0.2[b]	6.5±0.2[b,c]
n	4	5	4
Spleen			
Basal	6.0±0.3[b]	6.0±0.3[b]	5.3±0.2
20 min	7.6±0.4[b]	6.9±0.6	6.6±0.2[b,d]
n	5	5	3

[a] In controls, basal glucose levels and glucose levels at 20 min are 4.9±0.1 and 6.0±0.1 mmol/L, respectively (n=9). Values are mean ± SEM.
[b] Significantly higher ($P<0.05$) than controls.
[c] Significantly higher ($P<0.05$) for 10-µl grafts to the kidney versus liver.
[d] Significantly higher ($P<0.05$) for 10-µl grafts to the kidney versus spleen.

Basal glucose and insulin levels. At 1-month after transplantation, basal glucose levels in islet graft recipients were significantly higher than in controls, except for animals that received 10-µl grafts to the spleen (Tables 2 and 3). Basal insulin levels were significantly higher after transplantation to the liver and to the kidney than in controls for each graft size (Table 4). In contrast, basal insulin levels in rats with 2.5-, 5-, and 10-µl grafts to the spleen were similar to the basal insulin levels in controls.

Response to an IVGTT. Glucose infusion in control animals was associated with an immediate increase of the blood glucose levels that reached a plateau level after 15–20 min (Figs. 2–4). The insulin response showed a typical biphasic pattern,

TABLE 4. Basal insulin levels (average of the basal values before IVGTT and meal test, mU/L)[a]

Transplant site	Graft size		
	2.5 µl	5 µl	10 µl
Kidney	42.6±4.0[b,c]	38.0±2.3[b,e]	38.9±4.0[b]
n	6	6	4
Liver	40.7±6.0[b,d]	41.0±3.1[b]	36.9±1.5[b]
n	6	8	5
Spleen	24.3±2.5[c,d]	31.3±1.6[e]	29.3±2.5
n	6	5	5

[a] Basal insulin levels in controls are 27.4±1.4 mU/L (n=11). Values are mean ± SEM.

[b] Significantly higher ($P<0.05$) than controls.

[c] Significantly lower ($P<0.05$) for 2.5-µl grafts to the spleen versus kidney.

[d] Significantly lower ($P<0.05$) for 2.5-µl grafts to the spleen versus liver.

[e] Significantly lower ($P<0.05$) for 5-µl grafts to the spleen versus kidney.

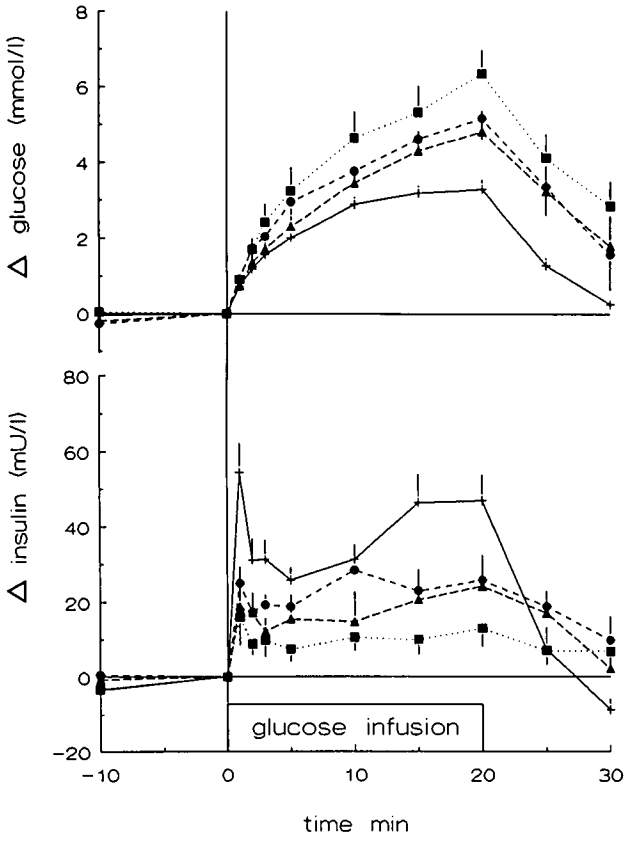

FIGURE 2. Incremental glucose and insulin response above basal values to IVGTT of controls (+) and of 10-µl (●), 5-µl (▲), and 2.5-µl (■) grafts to the kidney at 1 month.

i.e., an acute insulin response in the first minute followed by a second phase response after 5 min.

An acute insulin response to glucose infusion, although reduced, was seen 1 month after transplantation in all transplant groups (for kidney, liver, and spleen, shown in Figs. 2, 3, and 4, respectively). Glucose levels at 20 min were significantly higher after transplantation than in controls, irrespective of the graft size or transplant site (Table 2). Only in

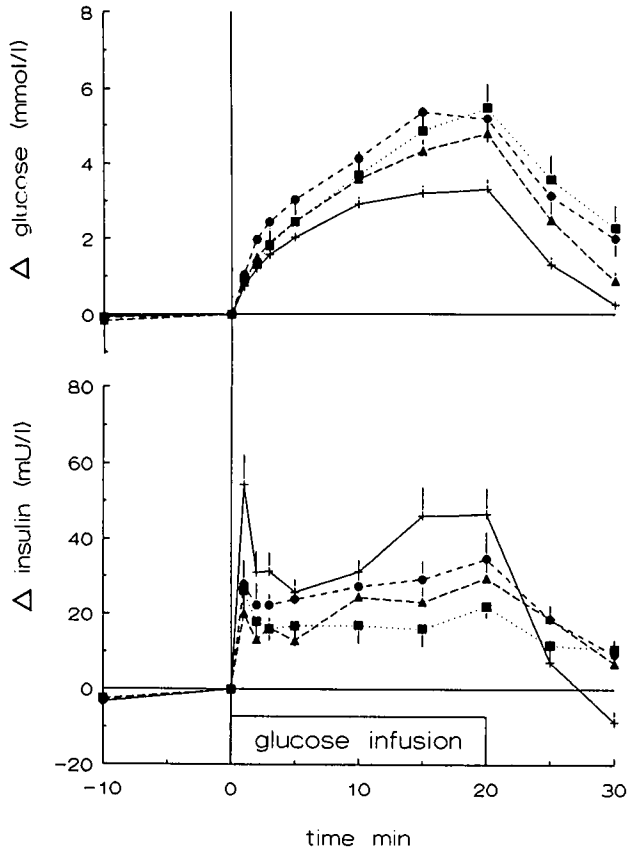

FIGURE 3. Incremental glucose and insulin response above basal values to IVGTT of controls (+) and of 10-µl (●), 5-µl (▲), and 2.5-µl (■) grafts to the liver at 1 month.

animals with grafts to the spleen did the glucose level at 20 min decrease with increasing graft size (Table 2).

The total insulin secretion 1 month after transplantation, determined by calculating the AUC-ins (0–20), was always lower in transplanted animals than in controls (Fig. 5). For each transplant site, the AUC-ins (0–20) increased with increasing graft volumes. This relationship was statistically significant for the liver ($r=0.43$) and for the spleen ($r=0.68$). For each graft size, the AUC-ins (0–20) was highest in rats with grafts to the liver and lowest for grafts to the spleen. The AUC-ins (0–20) of the insulin response curves of tests performed at 2 months were usually similar to or somewhat higher than the AUC-ins (0–20) at 1 month for each graft size and site, but the differences were not significant.

Response to a meal test. In controls, blood glucose levels were seen to increase above basal values 3 min after the beginning of the meal test and reached a maximum after 15–20 min (Figs. 6–8). A preabsorptive insulin response, i.e., an increase of the insulin levels preceding the elevation of the glucose levels, was observed at 1 min. Peak insulin levels were attained at 20 min.

Glucose levels at 20 min in response to a meal test performed 1 month after transplantation were significantly higher in all transplanted animals than in controls (Table 3). A tendency to decreasing glucose levels with increasing graft size was seen after transplantation to the liver and spleen,

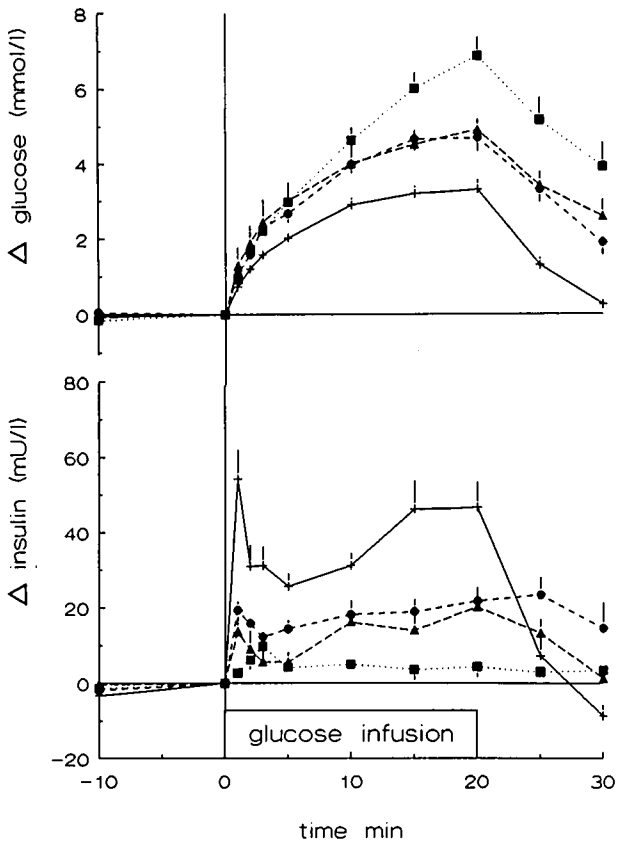

FIGURE 4. Incremental glucose and insulin response above basal values to IVGTT of controls (+) and of 10-µl (●), 5-µl (▲), and 2.5-µl (■) grafts to the spleen at 1 month.

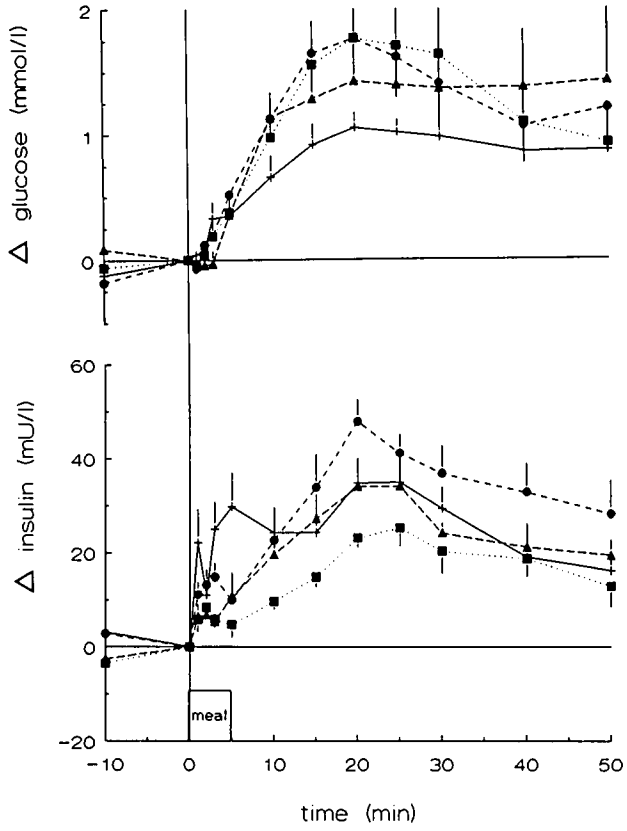

FIGURE 6. Incremental glucose and insulin response to a meal test of controls (+) and of 10-µl (●), 5-µl (▲), and 2.5-µl (■) grafts to the kidney at 1 month.

but not to the kidney. For each graft size, glucose levels were usually highest for grafts to the kidney.

The profiles of the insulin response curves show a reduced preabsorptive insulin response and a reduced insulin secretion during the first 5 min after initiation of food ingestion in all transplanted animals (for grafts to kidney, liver, and spleen, shown in Figs. 6, 7, and 8, respectively). Thereafter, peak insulin levels reached were similar to or even higher than observed in controls. At 1-month after transplantation, the AUC-ins (0–20) tended to increase with increasing graft size for grafts to the kidney and spleen but not for grafts to the liver (Fig. 9). The relationship between graft size and insulin output was statistically significant for the kidney (r=0.68) and for the spleen (r=0.59). The AUC-ins (0–20) was significantly lower than in controls only for the 2.5-µl grafts to the kidney and to the spleen. The AUC-ins (0–20) of the insulin response curves of tests performed 2 months after transplantation were only statistically different from the AUC-ins (0–20) at 1 month for 2.5-µl grafts to the kidney and for 10-µl grafts to the spleen.

DISCUSSION

This study in rats shows that grafts comprising ≥ 2.5 µl of highly purified islet tissue consistently induce the return to normoglycemia after isogenic transplantation under the kidney capsule, into the liver, or into the spleen in streptozoto-

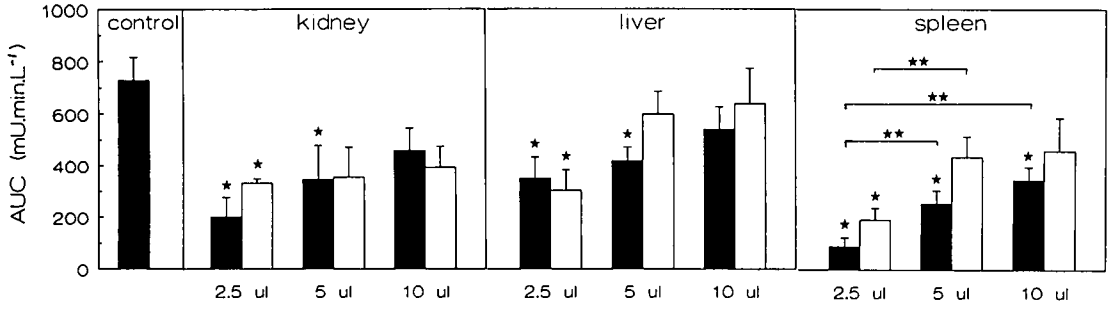

FIGURE 5. AUC-ins (0–20) in response to IVGTT at 1 month (■) and 2 months (□) after transplantation. AUC-ins (0–20) of control animals is 728±88 mU·min·L^{-1} (mean ± SEM). Significantly different (P<0.05) ★ from controls and ★★ between indicated groups.

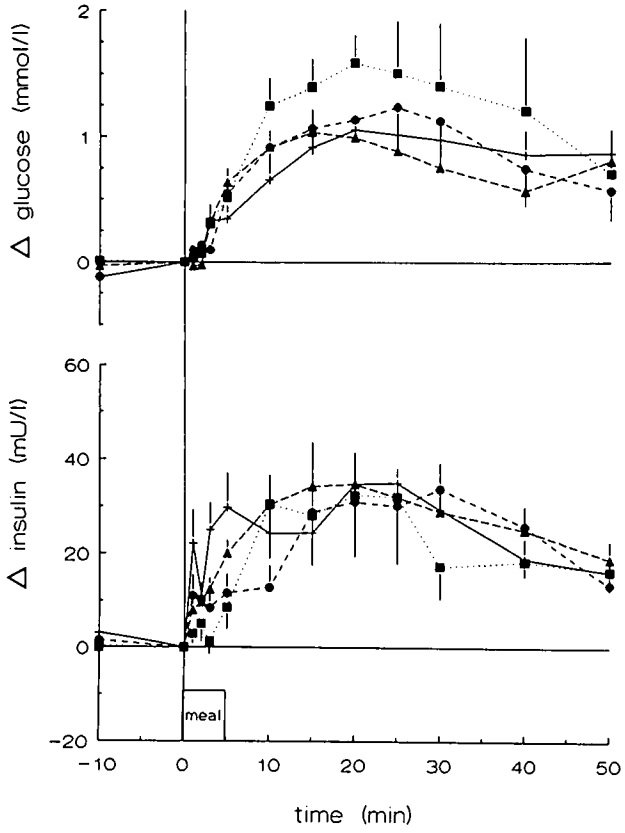

FIGURE 7. Incremental glucose and insulin response to a meal test of controls (+) and of 10-μl (●), 5-μl (▲), and 2.5-μl (■) grafts to the liver at 1 month.

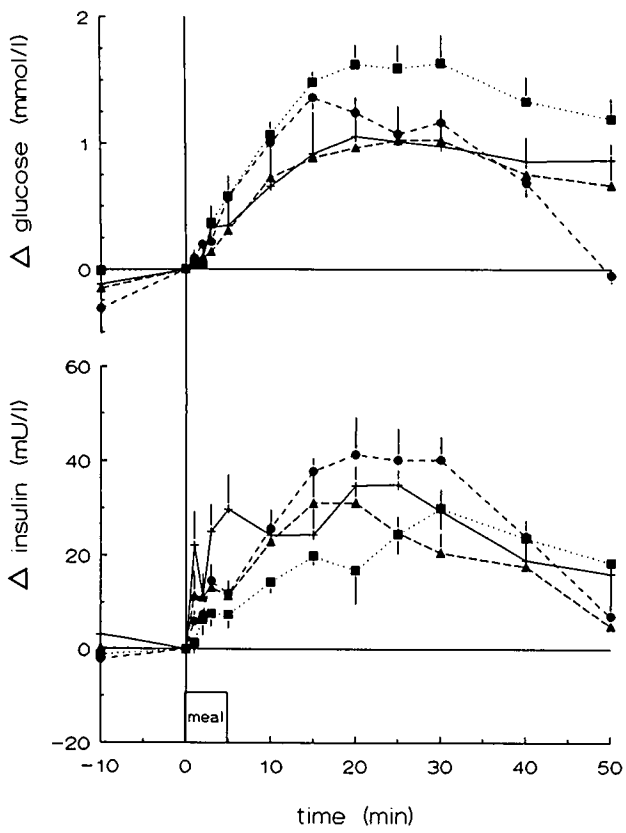

FIGURE 8. Incremental glucose and insulin response to a meal test of controls (+) and of 10-μl (●), 5-μl (▲), and 2.5-μl (■) grafts to the spleen at 1 month.

cin-diabetic recipients. The success rate of 1.25-μl islet grafts was found to be low, irrespective of the transplant site. We have shown previously by point count morphometry (*17*) and subsequently confirmed by insulin content determination (*12*) that the adult rat pancreas contains approximately 10 μl of endocrine tissue. Thus, a graft volume of 25% or more of the islet mass present in the native pancreas consistently results in normoglycemia. The minimal graft volume to restore normoglycemia is probably somewhat lower since success was occasionally observed with 12.5%.

This suggestion is corroborated by Keymeulen et al. (*23*), who found that normoglycemia was restored by intrahepatic rat islet isografts comprising 16% of the total pancreatic insulin content. Our findings in rats are also compatible with those obtained in dogs by Warnock and Rajotte (*4*). They found the minimal volume of intrasplenic islet autografts to be 4–5 μl of islet tissue per kilogram of body weight in mongrel dogs of 18–25 kg. This can be estimated to be 15–20% of the endocrine volume of the pancreas, since, in our laboratory, the pancreas of the mongrel dog of a similar size weighs 50–60 g and contains approximately 1.1% of islet tissue (unpublished observations). The above considerations indicate that the minimal graft volume to restore normoglycemia can be taken to be larger than 12.5% and probably somewhat lower than 25%.

This estimate is 2–4 times higher than the 5–10%, which reportedly is the minimum remnant after near-total pancreatectomy to maintain normoglycemia in rats (*24*), dogs (*25*, *26*), and man (*27*). One of the possible causes of this difference may be a reduced islet cell viability as a consequence of our islet isolation procedure, although these islets respond adequately to glucose stimulation in vitro (*19*, *28*). Theoretically, this difference could be explained by incomplete engraftment. However, this cannot be more than a contributing factor, since it is unlikely that engraftment is restricted to 50% or less of the transplanted islet tissue in view of the similar insulin content of islet grafts before and after transplantation, as reported by Keymeulen et al. (*23*). Another contributing factor may be the absence of sufficient regulatory mechanisms, such as parasympathetic innervation, although this influences the quality of insulin release rather than the maintenance of basal normoglycemia. Nevertheless, these factors together actually may offer sufficient explanation, since the suggested difference between the minimum grafted and the minimum native endocrine volume for maintaining normoglycemia may in fact be much smaller than 2–4 times as a consequence of a substantial regeneration of the remnant pancreas after near-total pancreatectomy (*24*).

In our study, the relationship between graft size and graft performance was assessed by using grafts of 3 different well-defined endocrine volumes, i.e., 2.5, 5, and 10 μl. Previous findings of others (*5*, *23*, *29*) were further extended by testing the performance not only by an intravenous glucose challenge, but also by the more physiological oral test meal. We found a usually outspoken reduction in the quantified insulin response after intravenous, but not after oral, stimulation.

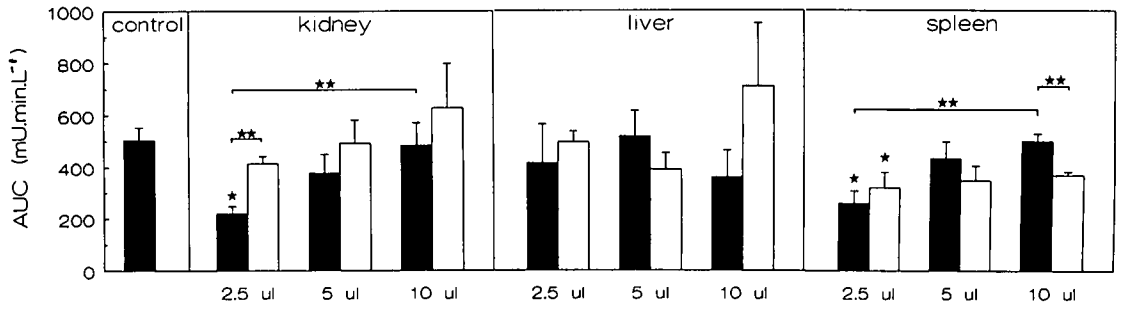

FIGURE 9. AUC-ins (0–20) in response to a meal test at 1 month (■) and 2 months (□) after transplantation. AUC-ins (0–20) of control animals is 506±48 mU·min·L^{-1} (mean ± SEM). Significantly different (P<0.05) ★ from controls and ★★ between indicated groups.

The high glucose levels during intravenous testing induced a maximum insulin output that was lower than the maximum capacity of controls. However, in response to the lower, more physiological, glucose levels during oral testing, the grafts gave similar quantified responses as controls. This indicates that high, but not low, stimulating glucose levels are associated with differences in glucose-insulin dose-response relationship between islet grafts and native islets. The most prominent difference between both testing methods is that during oral testing not only glucose but also indirect cephalic stimulation and incretin serve as insulin stimulatory factors (*15, 30, 31*), while this is only glucose during intravenous testing. The absence of direct cephalic stimulation may explain the reduced early insulin response during the first 5 min after food ingestion (*16, 32*). This, consequently, results in higher glucose levels, explaining the increased insulin levels during the second phase of the meal test, as also shown in humans (*33*).

Our study confirms that graft performance is better with increasing graft size (*5, 23, 29*), and demonstrates that this holds for each site tested. Nevertheless, some differences were observed among sites. Higher basal insulin levels than in controls were seen in animals with grafts to the kidney and liver, but not in animals with grafts to the spleen. For grafts to the kidney, this can be explained by drainage of insulin to the systemic instead of portal venous system. For grafts to the liver, we (*12*) and others (*24, 34*) have suggested that this is due to a reduced hepatic extraction of insulin by only a part of the liver tissue. Thus, the spleen location seems to have some advantage, since basal normoglycemia has been observed in association with normal basal insulin levels. Moreover, this is the more physiological route of insulin delivery.

Two months after transplantation, the areas under the insulin response curves were similar to the AUCs at 1 month, irrespective of graft size and graft site. Actually, especially with the smaller graft sizes to the kidney and spleen, there was some indication of improvement of graft performance. Thus, within our observation period, we found no indication of exhaustion of islets in animals receiving a minimal graft volume (*3*); however, this may well be detectable only after a longer observation period (*35*).

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ROLE OF ADENOSINE IN PRESERVATION BY THE TWO-LAYER METHOD OF ISCHEMICALLY DAMAGED CANINE PANCREAS

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The purpose of this study was to clarify the role of adenosine in preservation of ischemically damaged pancreas by the two-layer (Euro-Collins solution [EC]/perfluorochemical [PFC]) method using a canine model. Twenty-four-hour preservation of the pancreas graft subjected to 60-min warm ischemia was successful by the two-layer (EC with adenosine/PFC) method (4/5, 80%), but neither simple cold storage in EC (0/5, 0%), nor EC with adenosine (1/5, 20%), nor the two-layer (EC/PFC) method (0/3, 0%) was successful. Tissue ATP concentrations at the end of preservation by the two-layer (EC with adenosine/PFC) method were significantly higher compared with the two-layer (EC/PFC) method (7.23±2.17 vs. 1.56±0.40 μmol/g dry weight, $P<0.01$). Studies with [2-^{3}H]adenosine demonstrated that only part of adenosine was converted to inosine, hypoxanthine, and adenine, whereas the remainder was incorporated into adenine nucleotides in the pancreas graft. In addition, hypoxanthine, inosine, and adenine did not substitute for adenosine.

We conclude that provision of adenosine to ischemically damaged pancreas during preservation by the two-layer (EC/PFC) method allows ATP synthesis within the graft via direct phosphorylation of adenosine. Metabolic processes vital to repair damaged cells and maintain cellular integrity can be maintained,

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